A PERFUSION STUDY OF THE MOVEMENT OF STRONTIUM ACROSS THE GILLS OF RAINBOW TROUT (SALMO GAIRDNERII)

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Strontium uptake by fresh-water and marine fishes has been previously studied by either feeding, immersing, or otherwise exposing the intact fish to the isotope, and counting the activity gained by the animal. These methods give an understanding of the gross over-all uptake and elimination of the isotope, but offer little or no comprehension of the function of the gills or the mechanisms involved.

Preliminary feeding and immersion studies in our laboratory indicated that the gills of the rainbow trout were a major pathway for the uptake of strontium from water, although strontium was not concentrated in the fish to any great degree. In addition, the gills of fish are known to have an important role in homeostasis by their ability to actively take up some ions and to excrete metabolic products. Therefore, it was decided to investigate the rates of influx and outflux of strontium across the gill membrane as a first step in the study of the metabolic pathways and regulation of incorporated strontium in fish.

Keys' (1931) basic method for the perfusion of the gills of the eel was adapted for rainbow trout. The results obtained by this method suggest outflux rates compatible with the relatively poor ability of fish to concentrate strontium in the body from the water or food.

MATERIALS AND METHODS

A diagram of the gill perfusion preparation is presented in Figure 1. The internal system consisted of a perfusion pump to force a modified Ringer's solution through the gills, out the dorsal aorta, and into a reservoir. The pump simultaneously drew the fluid from the reservoir into a scintillation detector from which it was recirculated through the gills. The scintillation detection system consisted of a well-type crystal which contained a coil of small-bore glass tubing through which the perfusate flowed. The output of the detector was fed to a pulse height analyzer focused on the .513 mev peak of Sr^{85}. The signal from the analyzer was integrated by a precision count rate meter and recorded on a Sanborn four-channel recorder.

In addition to isotopic concentration, the flow, volume, and pressure of the internal system were recorded electronically. Flow and volume were recorded as hydrostatic pressure in the reservoir (Fig. 1, g). Efferent flow from the gills returned to this reservoir via an automatic syphon (Fig. 1, f) which emptied when

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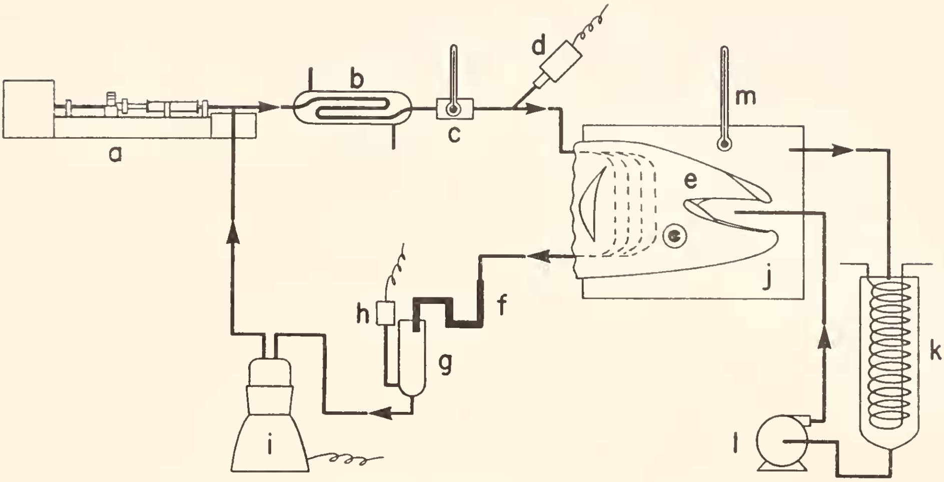

FIGURE 1. Diagram of gill perfusion preparation. Internal system: (a) perfusion pump, (b) cooling coil, (c) thermometer, (d) electronic pressure transducer, (e) fish, (f) automatic syphon, (g) reservoir with sidearm and pressure transducer (h) to measure volume and flow, (i) scintillation detector. External system: (j) diaphragm tank, (k) reservoir with cooling coil, (l) pump, (m) thermometer.

it attained its full capacity of 2.8 ml. This addition to the reservoir caused a measurable increase in hydrostatic pressure, and by use of the time scale on the electrogram, flow could be calculated. By the same means, an over-all decrease in hydrostatic pressure could be calibrated as a loss of volume in the internal system. Internal system pressure was measured as mm. Hg by a standard Sanborn medical pressure transducer (Fig. 1, d).

The Ringer's solution used had the following composition:

5.0 mEq K^+/l.
6.5 mEq Ca^{++}/l.
109 mEq Na^+/l.
112 mEq Cl^-/l.

This particular balance, which contains more Ca^{++} than frog Ringer's solution, had been found to support a trout's heart satisfactorily. Just before use, 0.4 g. $NaHCO_3$ and 0.9 g. glucose per liter of stock were added for buffer and nutrient.

The external system consisted of two liters of fluid which was pumped through the buccal and opercular cavities in order to maintain a good flow over the gills. Both the internal and external solutions were buffered in a like manner and aerated with 95% oxygen + 5% carbon dioxide gas so that the pH of the fluids remained at 6.5 to 6.7 throughout all experiments. Temperature was controlled in both systems at 13° C., an optimum for rainbow trout.

The fish was anesthetized in a solution of 0.2 g./liter of MS-222 (Tricaine Methanesulfonate, Sandoz) until respiratory movements ceased. After being weighed, the fish was placed in a holder and a median ventral incision was made from just anterior to the anus to about one centimeter posterior to the insertion of the pectoral fins. Then heparin was injected into the heart and the gastro-intestinal tract, liver, gall bladder, gonads, and air sac were removed. All main arteries, the air bladder, and the esophagus were ligated.

A cross-sectional incision was made in the mid-kidney to open the dorsal aorta, and the heart was fully exposed. The ventricle was separated from the atria and opened to receive a polyethylene cannula. The tip of the cannula was positioned in the bulbus arteriosus and tied. The time period from the start of the operation to this point was no longer than 6 or 7 minutes.

Once the gills were being perfused with the Ringer's solution, the dorsal aorta was cannulated by exposing the aorta at the site of the earlier incision in the kidney and inserting a snug-fitting polyethylene tube until its tip lay well in the region of the head kidney. Successful positioning was obtained by palpation of the kidney as the cannula was inserted. Once positioned, slight vacuum was applied to the open end to establish a good flow. This end of the cannula was kept about 5 cm. below the level of the fish, to avoid extreme pressure in the gills. Due to vascular resistance in the healthy gills a pressure of 45 to 75 mm. Hg was present in the afferent section of the system. Additional afferent pressure gained by the raising of the tip of the cannula will destroy the preparation.

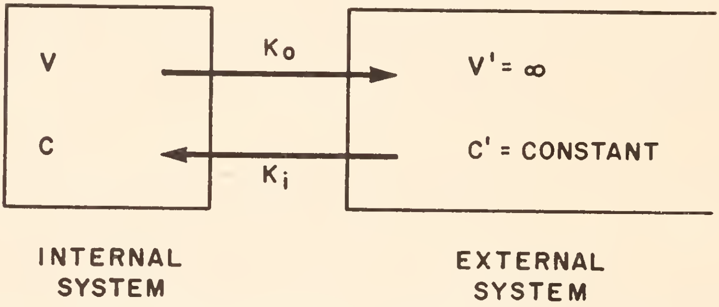

FIGURE 2. Model of two-compartment system as used in gill perfusion studies. C and C′ = concentration, V and V′ = volume.

Once the afferent and efferent flows were well established, the coelom was filled with a thick solution of gelatin just warm enough to be fluid. This solidified rapidly and sealed all bleeders. The fish's head was then inserted through the rubber diaphragm into the tank; the external system was filled with an appropriate fluid and circulation begun.

After the preparation had been completed and the blood flushed out of the gills, the flow from the efferent cannula was directed into the flow meter and recirculation was started.

The basic model of the preparation (Fig. 2) shows V′, the external volume, to be essentially infinite and C′, the external concentration, to be constant, due to the comparatively large volume of the external system and the short period of data collection. Actual measurements of concentrations in this system before and after experiments proved these assumptions to be valid.

Errors caused by volume changes due to osmotic forces or leakage from the internal system were difficult to evaluate. Leakage through the gills was found to be the most serious. By minimizing the time period during which data were collected it was felt that these errors would have no serious reflection on the basic conclusions.

Keys (1951) fully discussed the difficulties encountered in this type of experimentation.

RESULTS

Data acquired by this experimental method, using strontium-85 as a tracer ion, were considered as a linear regression of the internal concentration against time. Although the extended curve from transfer data of this type would be expected to be exponential, it was discovered that the linear treatment would introduce little error at this point and eliminate much of the difficulty encountered in endeavoring to handle both the influx and outflux with the same mathematical approach.

The equations

$$\frac{C_t \times V}{t \times A \times C'_o} = I \tag{1}$$

and

$$\frac{C_t \times V}{t \times A \times C_o} = O \tag{2}$$

were applied to the data for calculation of the influx rate (I) and outflux rate (O). C_t is the internal concentration at time t. C'_o and C_o are the initial external and internal concentrations, respectively. V is the initial internal volume, and A is the area of the gills. The area of the gills in relation to body weight was estimated from Price's (1931) figure of 2.2 cm.^{2} of gill area/kg. of body weight, which was determined on the black bass, *Micropterus dolomicu*. Although species differences probably exist, the error is constant throughout this study. I and O, the flux rates, have the dimensions of velocity, *i.e.*, cm./sec., and are similar to the permeability constant (Davson, 1951).

TABLE I

Influx rate (I) for strontium transport through the gill of rainbow trout

Exp. no.	Nutrient Ringer's solution internal concentration at t = o (μg. Sr^{++}/ml.)	Buffered distilled water external concentration at t = o (μg. Sr^{++}/ml.)	I (cm./sec.)
1	0	Sr^{85} (trace carrier)	1.1×10^{-8}
2	0	Sr^{85} (trace carrier)	1.3×10^{-8}
3	0	Sr^{85} (trace carrier)	2.6×10^{-8}
4	0	Sr^{85} (trace carrier)	1.0×10^{-8}
10	0	10 μg./ml.	0.5×10^{-8}
15	0	1 μg./ml.	5.2×10^{-8}
16	0	1 μg./ml.	12.0×10^{-8}
17	0	Sr^{85} (trace carrier)	7.7×10^{-8}
20	0	Sr^{85} (trace carrier)	15.0×10^{-8}

Mean ± Standard Error $5.2 \times 10^{-8} \pm 1.8 \times 10^{-8}$

Table II

Outflux rate (O) for strontium through the gill of rainbow trout

Exp. no.	Nutrient Ringer's solution internal concentration at t = o (μg. Sr^{++}/ml.)	Buffered distilled water external concentration at t = 0 (μg. Sr^{++}/ml.)	O (cm./sec.)
7	100 μg./ml.	0	1.1×10^{-5}
8	10 μg./ml.	0	1.1×10^{-5}
12	1 μg./ml.	0	1.8×10^{-5}
13	1 μg./ml.	0	1.3×10^{-5}
14	1 μg./ml.	0	1.4×10^{-5}
Mean ± Standard Error		$1.3 \times 10^{-5} \pm 0.1 \times 10^{-5}$	

In order to illustrate the treatment of the data received from this method, a protocol of a typical influx and a typical outflux is given. The results determined in similar experiments are listed in Tables I and II.

Determination of the influx rate

Experiment 1

Fish weight: 1094 g.

Estimated gill area: 2400 cm.^{2}

Sex: Male

Internal volume: 130 ml.

Internal medium: Nutrient Ringer's solution

External medium: Buffered distilled water plus trace amount of Sr^{85}Cl$_2$. This gave 625,000 cpm. (C′) in the scintillation detector.

Figure 3 shows the data from this experiment. Internal pressure, flow rate, and volume loss were used as physiological parameters of the condition of the gills. Extreme deviations in any one of these parameters would cause the experiment to be discarded.

By using the data from Experiment 1 in Equation (1) the value of I can be calculated:

$$\frac{130 \text{ cpm./cm.}^3 \times 130 \text{ cm.}^3}{2100 \text{ sec.} \times 2400 \text{ cm.}^2 \times 312{,}000 \text{ cpm./cm.}^3} = 1.1 \times 10^{-8}$$

cpm./cm.^{2}/sec./external concentration, or 1.1×10^{-8} cm./sec.

Table I shows the values of I determined in nine similar experiments. It can readily be seen that I appears to be independent of the initial concentration of the external system. Although the flow rate through the gills may or may not affect the rates developed here, every effort was made to keep this constant and all experiments were run at flow rates of 3.5 to 4.5 ml./min.

Determination of the outflux rate

Experiment 7

Fish weight: 1598 g.

Estimated gill area: 3500 cm.^{2}

Sex: Female

Internal volume: 80 ml.

Internal medium: Nutrient Ringer's solution plus 100 μg. Sr^{++}/ml. labeled with Sr^{85}. When circulated through the detector a count rate of 72,000 cpm. was obtained, corresponding to 360 cpm./μg. Sr^{++}.

External medium: Glass-distilled water buffered with 0.4 g. $NaHCO_3$/l.

The change in the concentration of Sr^{++} in the internal medium was estimated from the count rate resulting from the Sr^{85}, and is shown in Figure 4.

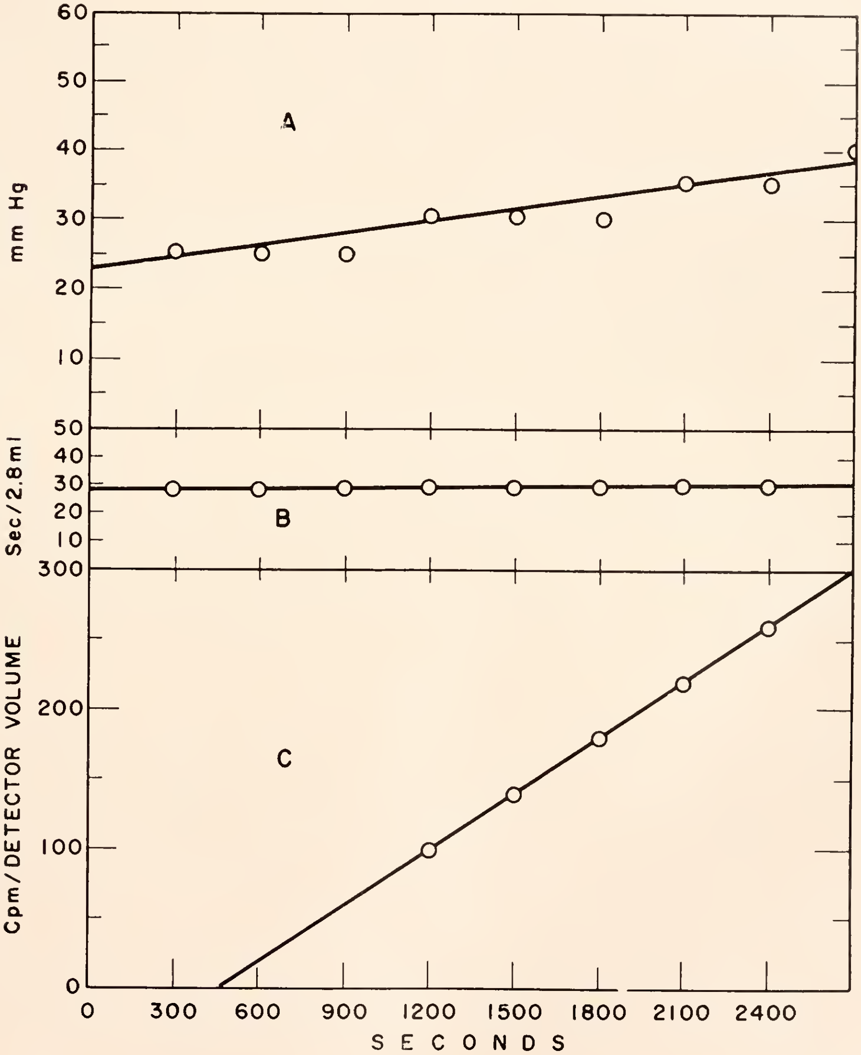

FIGURE 3. Data taken from original electrogram recorded during Experiment 1. (A) Internal pressure in mm. Hg; (B) flow in sec./2.8 ml.; (C) count rate due to Sr^{85} in cpm./detector volume. Points are discrete measurements from a continuous recording.

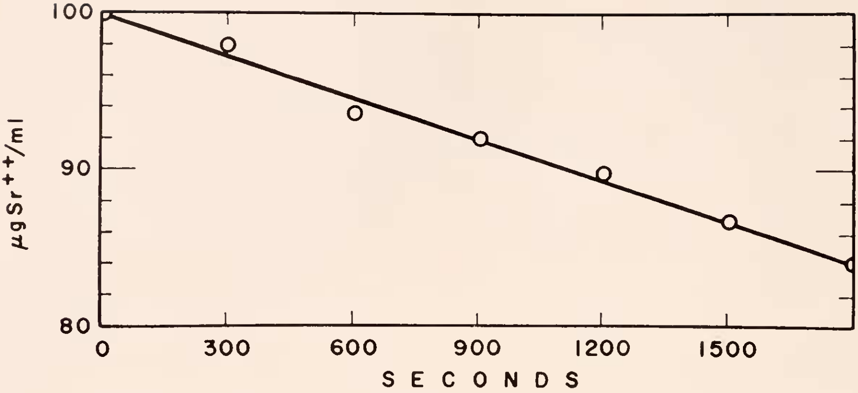

FIGURE 4. Internal concentration during course of Experiment 7.

In order to illustrate the fact that "C" can also be expressed in μg. Sr^{++}/ml., and to give some indication of the amount in μg. crossing the gill, the data from this experiment were expressed in μg. rather than in counts per minute.

The outflux rate can be calculated by Equation (2). The outflux rate for experiment 7 was 1.1×10^{-5} cm./sec.

Table II shows the O values determined in five similar experiments. Like I, O appears to be independent of the initial concentration.

DISCUSSION

Since the variance of O is greater than the variance of I, and the number of samples were unequal, Cockran's modification of Behrens-Fisher t test (Snedecor, 1956) was used and showed a marked difference between O and I at the 5% level.

If the rate at which the ion is transported through the membrane, as expressed by the flux rate, times the concentration of the ion, represents the amount that is transported through an area per unit time, then at equilibrium:

$$I\,C' = O\,C \tag{3}$$

It can readily be seen from the values of I and O obtained in this study that the concentration in the internal system at equilibrium will only be about 4% of the external concentration. In other words, Sr^{++} must be pumped out of the gills against a concentration gradient. In order to test this conclusion, an experiment was run in which both the internal and external systems contained the same concentrations of 1 μg. $Sr^{88} + Sr^{85}$/ml. It was found that the internal concentration decreased at an O of 2.2×10^{-5} cm. sec.

The apparent active transport can be explained in one of two ways. Either strontium is in direct competition with calcium in an active pump mechanism or it is a passive response to establish electrical neutrality, due to the active uptake of Na^{+} and other ions (Krogh, 1939). Further study of this transport mechanism, as well as Sr^{++} vs. Ca^{++} relationships, are now being undertaken.

Since fish in fresh water tend to excrete water and conserve salts, and experiments have shown that fresh-water fish will accumulate strontium (Cook, unpublished data; Saurov, 1957; Schiffman, 1959) from water at a ratio of approximately 1.5:1, there must be a mechanism to convert the ionized strontium to a non-diffusible form. Protein binding in the blood is just such a mechanism. Preliminary experiments in our laboratory, using both ultra-filtration of serum and dialysis of whole blood against Ringer's solution, indicate approximately 50% or more of the blood burden is in the non-dialyzable form, probably protein-bound.

Extravascular binding sites must also be taken into account since bone appears to bind strontium as tenaciously as calcium, although the rates of binding are probably much slower than those discussed here. The high outflux rate of diffusible strontium is also reflected in the relatively inferior ability of fish to concentrate strontium in the body.

A transport system for calcium, such as this postulated for strontium, would be ideal for fish such as the steelhead trout (*Salmo gairdnerii gairdnerii*) that migrate between fresh and salt water. A rate of protein binding faster than the O for Ca^{++} would allow for an uptake of Ca^{++} limited only by the availability of calcium in the environmental water, and the number of binding sites. Once the calcium content of the environmental water is raised beyond a certain degree the binding properties of the blood and extravascular sites would become saturated and the gill would become an organ of excretion of the excess diffusible portion of blood calcium. This mode of regulation would present a picture such as found by Houston (1959). He noted a definite increase in cellular calcium but a relatively stable plasma calcium in steelhead trout when adapting to sea water. Experiments are under way to test this hypothesis.

SUMMARY

1. A perfusion technique for the study of transport across the intact gill of rainbow trout is described.

2. It was found that the outflux rate of 1.6×10^{-6} cm./sec. was greater than the influx rate of 5×10^{-8} cm./sec. for strontium, and that strontium would go out of a fish gill against a concentration gradient under these experimental conditions.

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